

Studies on the Effect of Temperature on
the Embryo of Carinogammarus
mucronatus, Say

By

EMILY WALCOTT EMMART

A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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STUDIES ON THE EFFECT OF TEMPERATURE ON THE EMBRYO OF CARINOGAMMARUS MUCRONATUS, SAY

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SIX TEXT FIGURES AND ONE PLATE

I. INTRODUCTION

In studying the embryological development of the gammarid, *Carinogammarus mucronatus*, Say, it was noticed that the rates of development of the winter and summer embryos were markedly different. This, of course, would be expected from the difference in temperature of the two seasons. It was thought, however, that it would be interesting to see how the various functions of the developing embryo were affected specifically, by the change in temperature. Several phenomena were selected for study, including heart beat, peristalsis of the intestinal tract and liver lobes, and pigment formation in the eye.

The problem was carried on at the Johns Hopkins University and at the Marine Biological Laboratory, Woods Hole. The author wishes to acknowledge her indebtedness to Prof. H. S. Jennings and Prof. E. A. Andrews for their assistance and to Dr. Otto Glaser for the use of his constant temperature equipment at Woods Hole.

Material. The organism used for these experiments was a small brackish water gammarid, *Carinogammarus mucronatus*, Say, which is well distributed among the green seaweeds from Maine to Florida. It was originally found and

described by Say (1818) near the mouth of the St. John's River, Florida, and later found by Verrill (1874) in Vineyard Sound. I have also obtained specimens from Bar Harbor, Long Island Sound, and the estuaries of Chesapeake Bay. The most distinguishing characteristic of *C. mucronatus* is the presence of the single spine protruding along the mid-dorsal line from the posterior edge of each of the first three abdominal segments or pleons. The specimens used for these experiments were all obtained from the Eel Pond at Woods Hole, where they were found in abundance among the seaweeds, *Ulva* and *Enteromorpha intestinalis*, during the warmer seasons. Until the middle of December I collected them from washings of the mud of the Eel Pond near the shore. I also found them in rotting piles where they creep into empty barnacles and *Teredo* holes. This habit of burying themselves in the mud during colder weather must be generally characteristic since I was able to collect them all winter in the brackish water of the Magothy River (an estuary of the Chesapeake Bay), by washings from the mud of the bottom. Wherever collected they were always found in a region of green aquatic plants.

Technique. Mating pairs of gammarids were isolated in separate finger bowls, and were easily kept alive with frequent changes of sea water and with small pieces of old elm leaves, *Enteromorpha* or *Ulva* as food. As soon as the eggs were laid, the male was removed and the female allowed to swim about until the embryos had passed the onset of gastrulation. This seemed advisable since it was observed that eggs which were removed from the brood pouch soon after laying usually died at the critical stage of gastrulation, especially at the more extreme temperatures; while those remaining with the female usually survived. After this stage was passed the embryos were removed from the brood pouch and placed in separate vials. Once every 6 hours observations were made upon the eggs, the sea water changed, and the eggs turned over. At higher temperatures it was necessary to change the sea water more frequently. A 10-minute kymo-

graphic record was made every 6 hours from the commencement of peristalsis. A little later a similar record of the heart beat was started and both were run concurrently. The period dealt with is from the onset of peristalsis until hatching, a purely embryonic stage.

The apparatus used to maintain the eggs at a constant temperature consisted of a water bath in which was placed a thermal regulator. This controlled a small pump, which drew cold water from a frigidaire unit and forced it through coils suspended in the water bath. Two agitators kept the water of the bath in motion. The developing eggs were suspended in vials in the bath. The temperature of one experiment ($20.0^{\circ}\text{C}.$) was higher than that available with the water bath connected with the frigidaire unit, therefore a De Khotinsky constant temperature bath was used.

The above-mentioned systems maintained a constant temperature during the ordinary development. The following apparatus, however, assured constant temperature during the 10-minute observational periods. A Pfeffer cell was placed on the substage of the microscope, and the cold water from the bath circulated through it. The embryos were placed in a little sea water in a depression in the Pfeffer cell during observations. They were permitted to remain in this cell 10 minutes before the commencement of each record in order that they might recover from the slight shock of being removed. Since the room temperature was always above that of the water bath, it was not possible to keep the Pfeffer cell at exactly the same temperature as the bath. Nevertheless, the thermometer reading of the Pfeffer cell remained constant throughout the observations. The difference between the temperature of the glass substage and the developmental temperatures were $4.0^{\circ}\text{C}.$ at $12.5^{\circ}\text{C}.$ (bath temperature), $3.0^{\circ}\text{C}.$ at $14.5^{\circ}\text{C}.$, $2.0^{\circ}\text{C}.$ at $16.5^{\circ}\text{C}.$, $1.0^{\circ}\text{C}.$ at $18.5^{\circ}\text{C}.$, and $0.5^{\circ}\text{C}.$ at $20.0^{\circ}\text{C}.$, respectively. As the experimental temperature approached the temperature of the room the difference between the temperature of the bath and substage grows steadily less.

The range of the temperature used was from 12.5°C. to 20.0°C. (bath temperature). The lower limit of these experiments was governed by the fact that 12.5°C. was the lowest which could be secured with the available equipment. It is, however, a temperature far above lethal for *Carinogammarus*, which can stand almost freezing temperatures. The highest temperature used (20.0°C.) was the maximum temperature at which these eggs would develop under the existing laboratory conditions.

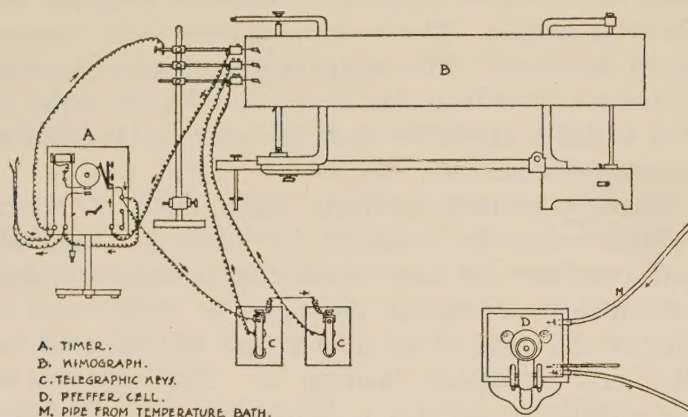


Figure A

The kymographic records were made on a continuous belt moving around two drums. Two arms were used for recording, one the movement of the heart beat, the other the start of a peristaltic wave. The arms were controlled respectively by signal keys on an electric circuit. One key was pressed at each heart beat, the other key was held down a second to mark the onset of peristalsis of the intestine but pressed and released immediately when recording the onset of peristalsis of the liver lobes. Time was marked on the record by an automatic Harvard timer controlling a third mechanical arm. The duration of each record of 10 minutes was controlled by a stop watch.

II. PERISTALSIS IN RELATION TO TEMPERATURE

While peristalsis of the alimentary canal has been studied in detail in higher forms, little is known concerning this phenomena among the invertebrates. As early as 1844 the contractility of the liver lobes in the crustacea was observed by Schlemm. This phenomena was later noted by Meckel (1846), followed by the description of the musculature of the liver lobes by Frey and Leukart (1847). A few years later Lereboullet (1853) and Weber (1880) also mentioned the contractility of the liver lobes, and the latter has given a full description of their structure in the gammarids, *G. marinus* and *G. fluviatilis*. Rossiiskaya and Pereyaslawzewa (1888) also noted briefly the 'contraction' of the walls of the intestine of gammarids. More recently Rogers ('27) reports in the adult *Daphnia*, "The distribution of the material in the intestine is affected by means of peristaltic and antiperistaltic movements. The movements involve not only the musculature of the intestine, but the so-called liver as well."

In *Carinogammarus mucronatus*, Say, this peristaltic activity is the first muscular contraction in the embryo. It consists of single wave-like contractions which are characteristic of both liver lobes and intestine. The first peristaltic contractions begin long before the intestinal tract is fully developed and at the moment when the buds of the liver lobes begin to push backward from the lobose fore-gut of the embryo. The primitive fore-gut and hind-gut are filled with green yolk material or deutoplasm, which was separated from the blastoderm during early development. Some of the larger yolk cells are still present in the lobose fore-gut, but these soon break down after the onset of peristalsis. The liver lobes arising as buds from the fore-gut require roughly about 12 hours at 16.5°C. for their development. At first they are a single pair of tube-like structures lying along the side of the primitive intestine. When fully extended they are almost as long as the intestine itself. Shortly before hatching these structures undergo a longitudinal fission resulting in two pairs of liver lobes, one pair on each side of the fully developed intestine.

According to Weber (1880) the liver lobes are covered with a 'tunica serosa' beneath which the muscle fibers are scattered in a network around the liver lobes. These muscles are similar to skeletal muscles in that they are cross striated. Beneath these muscle fibers lie layers of cells composed mainly of liver cells, secretory cells ('fermentzellen') and 'reservezellen.' Weber (p. 453) has also shown by the use of osmic acid, the presence of large fatty globules in the liver cells of *G. marinus*. I have also observed a similar condition in the liver lobes of *G. chevreuxi*.¹ Strings of fat bodies adhere closely to the outside of the tunica serosa (*G. marinus*, Weber, 1880, table XXXVII, fig. 1). Weber (p. 453) notes also that glycogen is formed by the cells of the fat bodies and the tunica serosa. If this latter statement is true, it may offer a possible explanation as to the source of energy for the activation of the muscle fibers of the liver lobes during the embryonic development.

The liver lobes or hepatopancreas of crustaceans are known to serve the two functions of food storage (Williams, '08) and secretion of digestive enzymes. Roaf ('06, '08) has made a number of investigations on the physiology of invertebrates and reports that in *Cancer pagurus*, tryptic starch-splitting, glycogen-splitting enzymes, also invertase, maltase and lactase are present in the digestive gland. Rogers ('27, p. 280) states that the fat-splitting enzyme, lipase, is found in *Homarus*, *Cancer pagurus*, and *Maja squinado*. In adult gammarids trypsin and pepsin are secreted by the 'fermentzellen' (Weber, 1880, p. 453).

In *C. mucronatus* the first peristaltic movement arises at the point of origin of the liver lobes. It is at first a slight contraction and occurs almost simultaneously in the intestine and the buds of the liver lobes. As the liver lobes develop, this contraction becomes a typical wave-like movement which, preceding backward, permits a gradual discharge of yolk

¹ The author wishes to acknowledge her indebtedness to Mr. R. Palmer formerly of the Marine Laboratory of Plymouth, England, for the slides of liver tissue of *G. chevreuxi*.

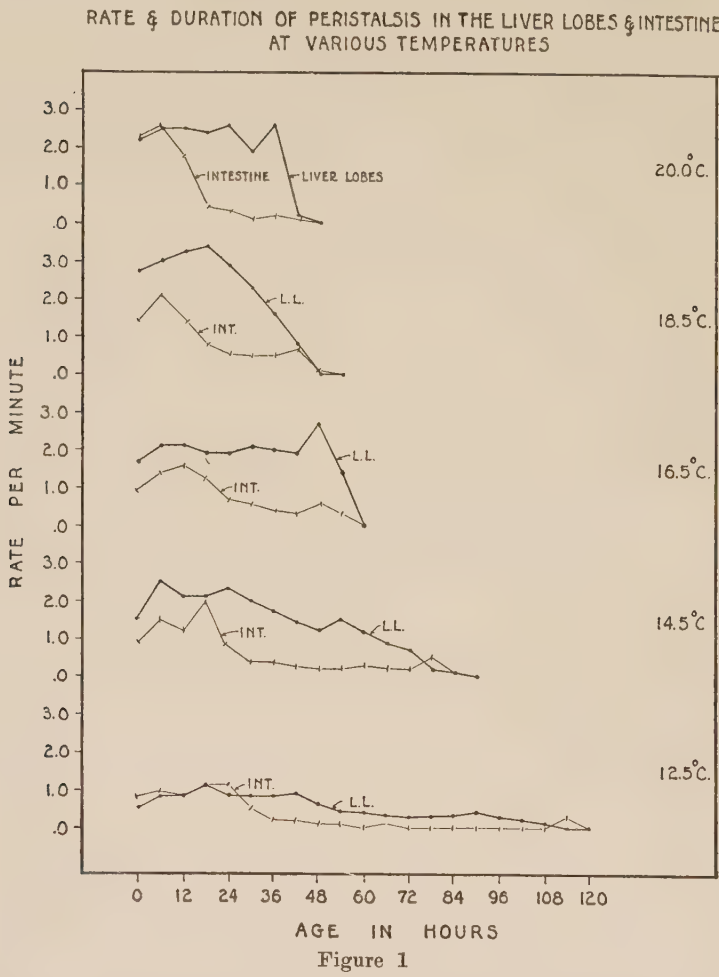
material into the intestine. This contraction of the muscle fibers of the liver lobes and intestine aids in the mechanical breaking down of the globular, fatty substances which they contain. As development proceeds, more and more of the original supply of yolk in the intestine is used up and more is received from the liver lobes. In the process of digestion the globules and granules of yolk disintegrate, leaving a clear greenish fluid which gradually becomes yellow and disappears altogether, so that the animal is perfectly transparent at the time of hatching. This greenish yellow color returns after the adult begins to feed.

The peristaltic wave in the intestine is not synchronous with that of the liver lobes. Nevertheless, both structures have the following interdependence; namely, that as the liver lobes grow backward the peristaltic wave in the intestine proceeds backward; and not until the liver lobes have reached their maximum growth does the wave proceed to the end of the intestine. This similarity of physiological behavior in the two structures closely parallels their similarity of origin.

Peristalsis occurs in the liver lobes only during embryonic development. The backward peristaltic wave in the intestine ceases, but a new wave of opposite direction starts at the posterior end of the intestine shortly before hatching. This metachronic wave lasts through adult life. At the onset of peristalsis in the embryo, the intervals between waves are irregular, the waves usually occurring in groups of two or three followed by a rest period. There is no definite rhythm, since the length of the intervals between waves is very irregular.

Rate of peristalsis. A study of the rates of peristalsis of the liver lobes and the intestine at all temperatures has shown the interesting phenomenon that the rates of activity of these two structures are consistently different—the liver lobes maintaining a higher rate of contraction than the intestine (fig. 1). The difference in rate between the two structures is, therefore, not a function of the temperature but a distinct difference in the physiological activity of the two structures.

The course of peristalsis, indicated by the slope of the curves (fig. 1) differs considerably in the two structures. In the former, at the three higher temperatures, a high rate of peristalsis is maintained for a long while, and then falls suddenly



just before hatching. At the temperatures of 12.5°C. and 14.5°C. the decrease in rate is gradual. In the intestine, however, it starts at a low rate, climbs to a peak, and immediately declines to a very low level. This low level is

maintained until a slight acceleration occurs just previous to the time of hatching (fig. 1). The actual rate at any stage for either organ is dependent upon the age and the temperature.

Discussion. The marked difference in rate of the liver lobes and intestine at the various temperatures as well as the fact that peristalsis occurs only in the former structures during embryonic development, seems to indicate that the activity of these structures may depend not only upon age and temperature but upon the quantity and nature of deutoplasmic substances which they contain. As has been previously stated this yolk material contains large quantities of fatty substances which are taken up by the liver cells. Rogers ('27) reports that in the adult *Daphnia* absorption of fat globules takes place either in the diverticula or in the anterior third of the intestine. These fatty substances may be converted into glycogen first or stored in the muscle tissue as fats to be converted into glycogen only after the utilization of carbohydrates of the deutoplasm. This storage and utilization of fats by muscle tissue has been reported in more highly specialized forms. Green ('26) has shown that in fish, fats may be stored in the muscle fibers themselves. Krogh and Lindhard ('20), Campbell, Douglas and Hobson ('21), Himwich, Loebel and Barr ('24) and others have shown that in man, fats are utilized by muscle after they have been converted into carbohydrates. Furusawa ('25) has also added that fats are utilized after the normal supply of carbohydrates is exhausted. The work of Krogh and Lindhard ('20) brought out the additional fact that the utilization of fats after the available source of carbohydrates is exhausted is accompanied by a marked decline in muscular efficiency. The work of Burn and Marks ('26) is also of special interest since they were able to show that excised liver tissue forms sugar from some source that is not protein, carbohydrates or lactic acid, which suggests that it is probably fat. It is to be recalled that Weber (1880) reports that glycogen has been found in the tunica serosa of the adult

gammarid. It would not be improbable, therefore, that the fatty substances stored in the liver lobes during embryonic development may be definitely related to the muscular activity of these structures. At any event, their gradual reduction in size and the lowering of the rate of peristalsis with its final cessation as hatching time is approached, corresponds directly with the observations on the disappearance of yolk substances in the liver lobes.

Rate of peristalsis

The effect of diverse temperatures can best be shown if the activity of the two structures is considered separately. In figure 2 are plotted the rates per minute for the intestine (part A) and for the liver lobes (part B). The width of each figure represents the rate in relation to age and to temperature. Part A of figure 2 shows clearly not only the increase with temperature in rate of muscular activity of the intestine but also its effect upon the course and duration of peristalsis. Peristalsis in the liver lobes (part B) is much more irregular than in the intestine. It is only when we compare the rate at 12.5°, 16.5°, and 20.0° that the effect of higher temperatures is shown. The slight rise in the rate of peristalsis of the intestine just previous to hatching tends to be obscured in the liver lobes.

Summarizing the results obtained from the study of peristalsis at various temperatures during the embryonic development, the following facts were obtained: 1) The rate of peristalsis is consistently higher in the liver lobes than in the intestine. 2) In the course of development the rate of peristalsis in the intestine rises and then declines rapidly, on the other hand the rate in the liver lobes while more irregular, nevertheless maintains a higher rate for a longer period. 3) Peristalsis ceases in the liver lobes at hatching but continues in the intestine at a very slow rate. 4) As was to be expected the rate of muscular activity in both structures is increased by higher temperatures.

RATE OF PERISTALSIS PER MINUTE AS AFFECTED
BY VARIOUS TEMPERATURES

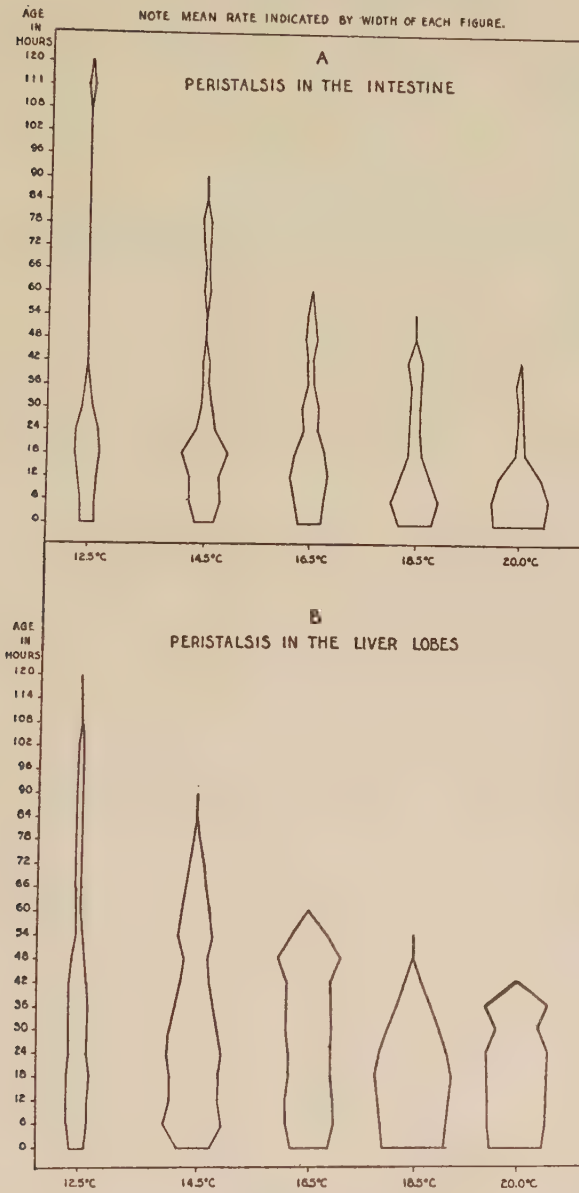


Figure 2

III. HEART BEAT IN RELATION TO TEMPERATURE

The fully developed heart of *Carinogammarus mucronatus* resembles closely the hearts of a number of Crustacea. It is a typical tubular structure suspended in a venous sinus or pericardial sinus from which the blood enters the heart through three pairs of ostia. The adult heart of *Carinogammarus mucronatus* extends from the first segment to the sixth, terminating as the median dorsal posterior aorta. At the anterior end the blood flows forward through the anterior aorta and two facial arteries which extend forward on each side of the median line. The blood passes into the heart through the three pairs of venous ostia which are closed by valves during systole. The adult heart muscle of *C. mucronatus* is of striated muscle fibers as is also the case in *Homarus vulgaris* and *Palinurus vulgaris* (Ritchie, '28, p. 40).

Little is known regarding the embryonic hearts of the Arthropods in general. Clark ('27, p. 12) states briefly that "it is possible that as a general rule in Arthropods the heart is laid down in the embryo as a tube of smooth muscle and that this is replaced by striped muscle." This is known to occur in *Limulus* and in this form the origin of the heart beat is myogenic since the accelerator and inhibitory nerves connect with the heart after the onset of heart beat (Carlson and Meek, '08). More recent work on the structure and behavior of the skeletal, the muscle fibers of the heart and of the amnion of the chick, in tissue culture, has shown that both smooth and striated muscle fibers are of themselves contractile (Lewis, '20). Therefore, the fact that a muscle is smooth or striated cannot be correlated with myogenic or neurogenic activity. Clark ('27, p. 14) states that except for the work on *Limulus* the present knowledge is insufficient to make any definite statement as to the myogenic or neurogenic origin of the heart contractions in Crustacea. In *C. mucronatus* the histological evidence is lacking as to the presence or absence of nerve fibers at the time of onset of the heart beat. The irregularity of the beat at the onset of heart muscle contraction is, however, in marked contrast to the regularity of contraction which occurs later.

The forerunner of the heart appears in *C. mucronatus* as a tube just posterior to the dorsal organ. Shortly before the heart begins to beat, large blood corpuscles can be seen drifting in the transparent blood plasm, moved about by the peristaltic movement of the intestine which lies directly below the heart. The first faint contractions of the heart take place just posterior to the dorsal organ. They are at first very irregular; the contractions being followed by periods of tetanus of irregular duration. As the heart develops these periods of tetanus show a tendency to rhythmic frequency. Later in development the rhythm is obscured by the rapidity of heart beat which has gradually increased as the hatching period is approached. Kymographic records of 10-minute duration made once every 6 hours from the onset of heart beat to hatching show the successive stages in the development of the heart. Plate 1 shows the records of 2-minute duration clipped from the record of 10-minute duration. Here is shown the graphic representation of the above-mentioned phenomena. The grouping of the beats, the increase in rate, the change in rhythm and the shortening of the duration of tetanus with increase in age appear to be characteristic of the embryonic heart of *C. mucronatus*.

Rate of heart beat. The irregular periods of tetany occur at the onset of heart beat at all temperatures. However, after the first brief period the rate of heart beat rises rapidly, the rate continuing to accelerate as the embryo increases in age. After hatching the beat is too rapid to count at high temperatures. In figure 3 is given the graphic record of the average rate of beat per minute plotted in 6-hour periods from commencement to the time of hatching. The accelerating effect of higher temperatures on the embryonic heart is markedly brought out by the data. In this figure no attempt has been made to correct the slope of the curve so that the irregularities may possibly be accounted for by experimental error.

The figure above shows that temperature affects the embryonic heart in three distinct ways. 1) The rate of increase

from hour to hour is dependent upon temperature. This is easily observed by noting the slope of the lines for the various temperatures. 2) The highest rate attained is also affected. If one case is taken from the many experiments it will perhaps clarify the situation. The highest rate of heart beat per minute recorded at 12.5° was 88; that at 14.5° ,

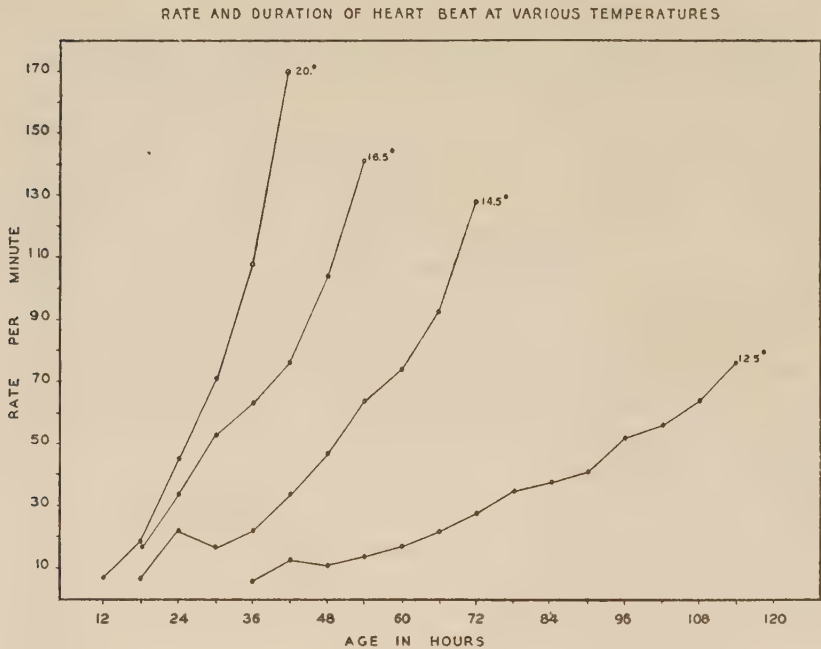


Figure 3

157; at 16.5° , 176; and at 20.0° , 190. The rate of heart beat, therefore, never reaches the height at 12.5° that it does at 20.0°C . 3) The rate of morphological development of the heart is affected by different temperatures. Since these experiments deal with developing embryos the time of onset of the heart beat will be affected by the various temperatures used.

IV. THE EFFECT OF TEMPERATURE UPON RATE OF DEVELOPMENT

Since the rate of either peristalsis or heart beat is a function of the age of the embryo as well as of the temperature, the effect of the temperature upon the rate of morphological development alone is of interest. The difficulty of selecting specific stages of development which could be readily recognized led to the selection of the onset of the development of eye pigment as the initial primary stage, the first heart beat as a second, the first jerky contraction of body muscles as the third, the deposition of melanin in the eye as a fourth, and the time of hatching as the last. Calculations in time in hours represent the interval between the onset of peristalsis and each of these succeeding phenomena. It must be kept in mind that the onset of these stages of development is based on continuous readings made once every 6 hours during the entire embryonic period. They are not marked by any sudden changes such as moulting stages, but represent a series of points in the continuous growth of the embryo, at which the onset of new activity occurred. The first eye pigment was taken as a specific stage in the morphological development of the eye, the onset of heart beat as the specific point in its development where the first feeble contractions of the heart begin. The body muscle contractions mark the first flexions of the embryo within the egg case, while the time of hatching represents the effect of various temperatures upon the time required for total development between the onset of peristalsis and hatching. The mean hours with their probable error (table 1) indicate the significance of the accelerating effect of the higher temperatures during successive stages of embryonic development.

Since the first investigations of Hertwig (1898) of a systematic and quantitative study of development at different temperatures numerous workers have also attempted to apply to biological phenomena Van't Hoff's formula for the behavior of chemical reactions. Using the equation,

$$\frac{K_2}{K_1} = e^{\frac{\mu}{2} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)}$$

Crozier ('24) was able to show that in a number of cases biological processes conform to this equation. According to this formula the μ value is a constant or temperature characteristic which represents reactions underlying the biological activity at a series of increasing temperatures. If the logarithm of the data is plotted against the reciprocal of the absolute temperature and the resulting graph is a straight line then the μ value is a constant and the equation of Arrhenius may be applied to the data.

TABLE 1
Mean time of morphological development in hours

TEMPERATURE 12.5°	14.5°	16.5°	18.5°	20.0°c.
1. Total age at hatching in hours				
114.50 $\pm$ 1.680 log. 2.05881	78.40 $\pm$ 2.180 1.89232	59.50 $\pm$ 0.905 1.77452	46.00 $\pm$ 0.984 1.66276	42.40 $\pm$ 0.040 1.62737
2. Onset of melanin				
84.83 $\pm$ 2.355 log. 1.92855	70.33 $\pm$ 0.485 1.84714	48.50 $\pm$ 2.170 1.68574	25.50 $\pm$ 3.561 1.40654	6.80 $\pm$ 1.034 0.83251
3. Body muscle contraction				
45.00 $\pm$ 0.733 log. 1.65321	27.80 $\pm$ 1.051 1.44404	24.66 $\pm$ 0.773 1.39199	16.60 $\pm$ 0.103 1.22011	14.50 $\pm$ 0.936 1.16137
4. Onset of heart beat				
37.33 $\pm$ 1.327 log. 1.57206	21.30 $\pm$ 0.946 1.33041	19.66 $\pm$ 0.439 1.29358	13.00 $\pm$ 0.972 1.11394	11.50 $\pm$ 1.337 1.06070
5. Onset of eye pigment				
18.15 $\pm$ 1.310 log. 1.25888	16.20 $\pm$ 1.085 1.20952	13.00 $\pm$ 1.101 1.11394	8.00 $\pm$ 0.756 0.90309	6.80 $\pm$ 1.034 0.83251

In the present problem the log of the data (table 1) for the intervals of time between onset of peristalsis and the series of growth stages previously mentioned were plotted against their corresponding reciprocal temperatures. Figure 4 shows that the onset of heart beat, body muscle contraction and time of hatching tend to fall in a straight line although the deviation from the curve make it impossible to calculate a constant μ value. Considerable fluctuation occurs in the data for the

deposition of the primary eye pigment and the deposition of the melanin pigment shows especially that there is a distinct change in rate of development of the pigment at high temperatures. Since it was observed in some individuals that the eye may develop without melanin during embryonic life this does not represent a phase of morphological development of the eye but rather a chemical change in a specific substance. Crozier ('24) has also shown that in a number of biological processes similar breaks in the graph occur at certain

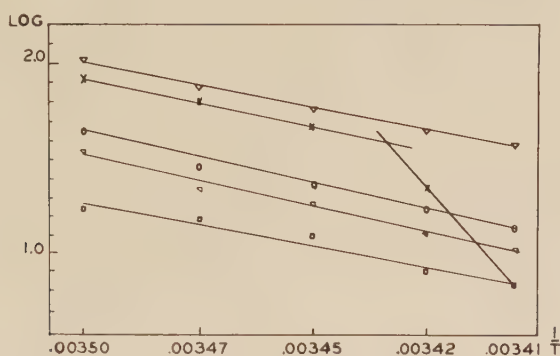


Fig. 4 The effect of various temperatures upon the time of morphological development at successive stages of development of the embryos of *Carinogammarus mucronatus*, Say. (The time required for development is plotted logarithmically against the reciprocal of the absolute temperatures.) ▽, time of hatching; ×, onset of melanin; ○, onset of body muscle contractions; ◐, heart beat; ◑, onset of eye pigment.

temperatures indicating, he believes, a difference in the chemical activity of the underlying biological phenomena.

In the attempt to express physiological activity as effected by change in temperature, Krogh ('14) and numerous other workers have used the modified equation of Van't Hoff formula,

$$Q_{10} = \frac{K_2}{K_1} \left(\frac{10}{T_2 - T_1} \right)$$

This equation attempts to express the rate of increase of chemical activity as affected by a series of temperatures. Van't Hoff (1884) was able to show that chemical reactions are either doubled or tripled for each 10° rise in temperature.

Krogh ('14) working with developing frog eggs was able to show that the Q_{10} values were not constant but decreased with temperature increase. Friend ('27) also applied the formula to the development of *Buccalatrix canadensiella* and obtained diminishing values of Q_{10} as the temperature increased. And Ludwig ('28) in studying the effects of various temperatures on the development of various stages of the insect, *Popillia japonica* Newman, also found that temperature characteristics were not constant but in some stages regularly increased and in others decreased with increase in temperature according to the stage of the life history. He concludes, "if temperature characteristics can be used as an index to the type of reaction underlying growth, the reaction is different for each stage of the life cycle and for the same stage it is different for nearly every temperature interval studied. . . ." He adds that "from these studies it appears that temperature characteristics of growth cannot be used in an analysis of its nature."

An analysis of the Q_{10} values of the present problem appears to bear out the findings of Ludwig ('28) for rate of development of organisms at various temperatures, namely, that with each stage of growth the temperature characteristic varies.

TABLE 2
Table of Q_{10} values

TEMPERATURE RANGE (C.)	1 TIME OF HATCHING	2 ONSET OF MELANIN	3 ONSET OF BODY MUSCLE CONTRACTION	4 ONSET OF HEART BEAT	5 ONSET OF EYE PIGMENT
12.5° — 14.5°	0.151	0.391	0.035	0.050	0.566
14.5° — 16.5°	0.224	0.156	0.549	0.670	0.332
16.5° — 18.5°	0.276	0.039	0.141	0.126	0.088
18.5° — 20.0°	0.579	0.00014	0.403	0.439	0.336

From the above table it will be seen that the data on the total time required for hatching on the various temperatures, bears out the theory of Van't Hoff that with every increase of ten degrees physiological reactions are either doubled or tripled. Here the increase is more than three times that of the lowest temperature over a temperature range of 7.5°C.

The data for the deposition of melanin show consistently decreasing factors for Q_{10} with increase in temperature. The difference between rate of activity in the deposition of melanin at high temperatures and rate of development of the organism shows that development of the melanin is influenced by factors other than morphological ones. The values of Q_{10} for the onset of body muscle contractions and heart beat, if corrected for the experimental error at 14.5°C . (fig. 4), will with increase in temperature read as follows: For body muscle contractions, $Q_{10} = 0.035, 0.163, 0.303$ and 0.403 and for onset of heart beat, $Q_{10} = 0.050, 0.131, 0.307$ and 0.439 . Thus it may be seen that with increase in temperature the Q_{10} values increase for morphological development as represented by time required for hatching, time of onset of body muscle contractions and time of onset of heart beat. From the curves in figure 4 it may be seen that onset of eye pigments tend to follow the same curve as that for the other stages of morphological development, but the experimental error appears in two points and therefore no attempt was made to correct for the Q_{10} values.

V. THE EFFECT OF TEMPERATURE UPON EYE PIGMENT

Considerable work has been done on the effect of temperature upon the deposition, duration and intensity of eye pigments of the gammarid, *Gammarus chevreuxi* (Huxley and Ford, '25; Ford and Huxley, '27; '29). In the normal wild type form of this gammarid a primary red pigment is followed by a deposition of the black pigment, melanin. Observations on the developing embryo of *Carinogammarus mucronatus*, Say have shown that the primary pigment to appear was yellow and that this became obscured by a red pigment, which in turn was hidden by a deposition of melanin. Therefore, while making the series of observations just previously described in sections II, III and IV, notations were made once every 6 hours of the onset and development of the eye. It was thought that by observing the developing eye at different temperatures it would be possible to determine:

First, the presence or absence of yellow pigment at all temperatures, and second, the effect of temperature upon the time of onset of these various pigments.

When the eye first starts to develop it appears externally as an irregular slit-like depression more or less pointed at the ends. As the ommatidia form, the eye broadens, gradually assuming an oval shape which it maintains all through the embryonic life and after the first moult. With the second and third moults the longitudinal axis of the eye merely increases but after the fourth moult the surface nearest the antennae begins to indent, the eye taking on more of the characteristic bean-shaped appearance with each successive moult.

When the ommatidia first make their appearance the five retinular cells are relatively very large, and the ommatidia, usually four or five in number, touch each other. Shortly before hatching these move apart, leaving an inter-retinular space between the ommatidia. This space develops a white pigment which is apparently similar to that described for *Gammarus chevreuxi* (Sexton and Wing, '16). By the time of hatching some eight or nine additional ommatidia make their appearance around the periphery of the eye. After hatching these move toward the upper surface as new ones develop around the margin, the number increasing with each successive moult—a mature animal having as many as seventy or eighty. At the time of hatching the five retinular cells containing the pigment of the eye give the ommatidia a pentagonal rosette shape. As the number of the ommatidia increase the ommatidia gradually become spherical.

The yellow pigment. The yellow pigment was observed to appear in granules around the developing cone cells before the walls of the retinular cells are distinct. Different groups of individuals reared at 12.5°, 14.5°, 16.5° and 18.5°C. showed the presence of the primary yellow pigment but in all four experiments the yellow pigment is of short duration, being soon hidden by a deposition of red pigment which makes its appearance about 6 hours later. As would be expected, from

12.5°, 14.5°, 16.5°, to 18.5° the onset of the yellow pigment coincides with the more rapid development of the eye. However, at 20.0° no yellow pigment was observed since melanin made its appearance at the very onset of pigmentation. The rapid onset of melanin at 20.0° may have hidden the yellow pigment or it may be of such short duration at this temperature that it escaped observation. The appearance of the yellow pigment at all lower temperatures indicates that it is normally present in the eye.

The red pigment. In the normal development of the Carinogammarus eye the second pigment to appear is red, but at the extreme temperatures of 12.5° and 20.0° variations occur. At 12.5° the onset of red pigment seems slightly hastened occurring in a few eyes at the very onset of pigmentation. At this temperature also the red as it darkens becomes a brilliant orange red quite different in appearance from the deep red eyes at the higher temperatures. At 14.5°, 16.5°, and 18.5°C. the red pigment follows the normal disposition of yellow. The bright red darkens into deep red which seems more brilliant at the lower temperatures. At 20.0°C. the red pigment observable in a few cases, was exceedingly transitory and was rapidly hidden by black, the melanin appearing in small granules at the very onset of pigmentation. In a few cases black appeared to be even preceded by chocolate color, the distinct red color only appearing around the periphery of the eye later. The chocolate color appears in this species only at high temperatures and then only at the very onset of pigmentation. It is possible that this might be due to the presence of the carotinoid substances at the same time as the melanin granules.

The gradual intensification and darkening of the red pigment differs considerably from that described for certain red-eyed strains of *G. chevreuxi* (Allen and Sexton, '17), in which red color darkens to chocolate and finally is replaced by melanin. In *Carinogammarus mucronatus* the red pigment before hatching gradually deepens from a light red through bright to dark red until finally it becomes hidden by a deposition of melanin in the most fully developed ommatidia in the

center of the eye. A few cases were observed at 14.5° and 16.5° in which the eyes were red at the time of hatching but melanin developed in these after hatching. In practically all other specimens, except those which developed at 20.0° , some red was clearly observable around the periphery at the time of hatching.

The black pigment. The appearance of the chocolate color in the eyes of *Carinogammarus mucronatus* occurred only in embryos developing at 20.0°C. at which temperature the development of melanin seems greatly hastened. This peculiar chocolate tinge was also observed in strains of *G. chevreuxi* by Huxley and Ford ('25), who stated that the "darkening of the scarlet to black is entirely due to the deposition of the insoluble melanin." In isolating genetically certain strains of red-eyed *G. chevreuxi* Huxley and Ford ('25) were able to obtain certain strains of 'slow' and 'rapid darkeners.' The 'slow darkeners' were a strain of 'faint brownish red eye forms' while the 'rapid darkeners' had eyes of deep chocolate color. Chocolate tinge appeared only in the eyes of *Carinogammarus mucronatus*, Say, when development was hastened by high temperature.

As has been previously shown, acceleration of deposition of melanin is very rapid at high temperatures. A better idea of the comparative effect of temperature upon onset of the pigmentation may be obtained if the data for yellow, red and black pigment is plotted in curves (fig. 5).

The similarity of the curves for yellow and red pigment as affected by the same temperatures may be considered as indicative of a chemical similarity while the steeper slope of the curve for black pigment indicates a more rapid deposition than that of yellow or red pigment. It is to be remembered that Verne ('20) and Palmer ('22) have disagreed as to whether yellow and red pigments of certain Crustacea are like those of plants, but they agree in stating that they are both carotinoid substances. Huxley states that melanin is insoluble in chloroform and alcohol, thus removing it from the carotinoid group (Ford and Huxley, '27). In studying

the rate of development of the melanin after extrusion in *G. chevreuxi*, Ford and Huxley ('29) have stated that the effect of temperature (10° to 13°C and 28°C .) on normal black-eyed forms is almost negligible. Except for a slight reddish tint in black eyes (10° to 13°C .) all those reared at 28°C . appeared black at extrusion from the brood pouch. Those reared at 10° to 13°C . became black a few days after extrusion. The present study shows that in *C. mucronatus* before hatching the degree of temperature has a marked effect upon the time of development of melanin. In addition it has been shown that

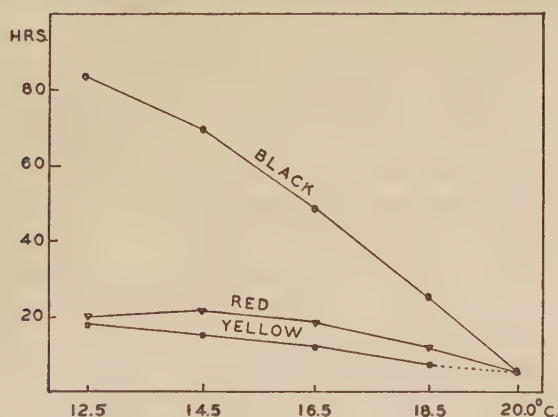


Fig.5 The effect of various temperatures on the time of deposition of eye pigments in developing embryos.

the same series of temperatures has a markedly different effect upon melanin and carotinoid pigments. The results are suggestive that this difference in reaction to increased temperatures may be correlated with the difference in the chemical nature of the two substances.

SUMMARY

The study of the effect of various temperatures upon the developing embryo of *Carinogammarus mucronatus*, Say has shown that,

1. The peristaltic movements of the liver lobes and the intestine proceed at a different rate in the two structures.

The rate in the liver lobes and the intestine rises slightly and declines as the yolk substances stored within the structures are utilized during embryonic development. By hatching time peristalsis has ceased in the liver lobes while it continues at a low rate in the intestine after hatching. With increase in temperature the rate of peristalsis is accelerated.

2. Heart beat commences shortly after peristalsis. It is irregular at first but gradually increases in rapidity. As heart beat increases periods of tetany appear. These however, are obscured as rate increases and cease to be observable just prior to hatching. As was expected, increased temperature had an accelerating effect upon the rate of heart beat.

3. The effect of various temperatures upon morphological development is best demonstrated by the length of time required for development between onset of peristalsis and hatching. This may be expressed by the equation,

$$Q_{10} = \frac{K_2}{K_1} \left(\frac{10}{T_2 - T_1} \right)$$

the temperature characteristics increasing with higher temperatures, an increase of nearly 75% over a temperature interval of 7.5°C. The plotting of the logarithm of the data against the reciprocal of the absolute temperature for the several stages of development prior to hatching, shows that the onset of heart beat and body muscle contractions tend to follow the curve for hatching.

4. The observations on deposition of eye pigment at various temperatures show that a yellowish pigment normally appears first; this is followed by red which in turn is ultimately hidden by a deposition of the black pigment, melanin. The rates of deposition of these pigments show that the deposition of melanin is greatly stimulated by high temperatures and that the differences in reaction to the same series of temperatures may be correlated with the chemical differences between carotinoid substances and melanin.

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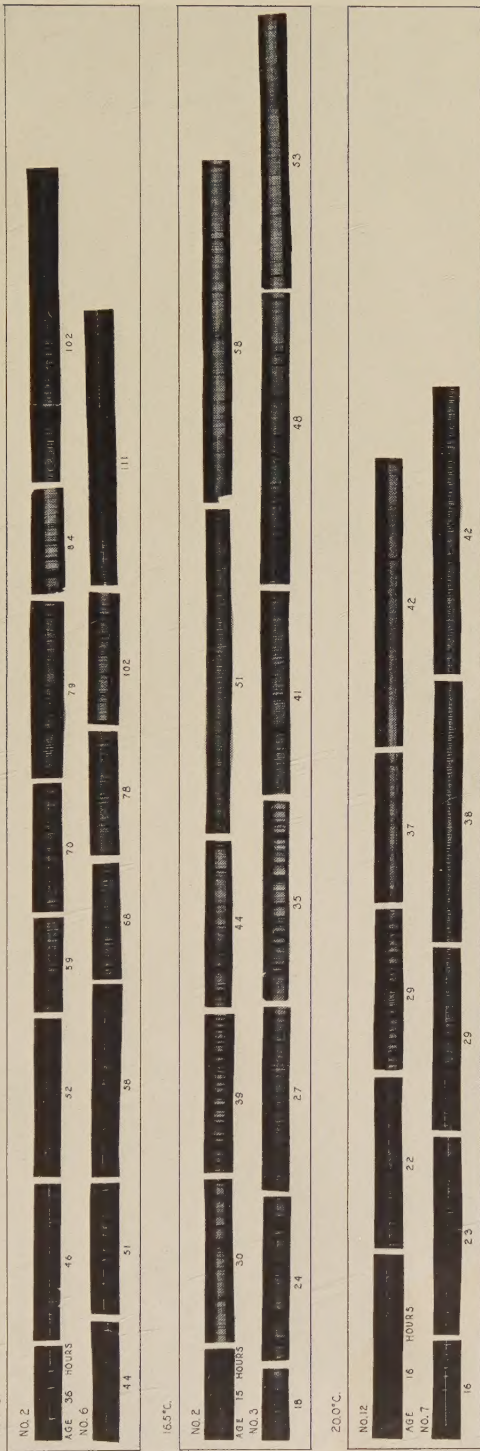
EFFECT OF TEMPERATURE ON CARINOGAMMARUS

EMILY WALCOTT EMMART

PLATE 1

TWO MINUTE RECORDS OF HEART BEAT OF INDIVIDUALS DEVELOPING AT DIFFERENT TEMPERATURES — SHOWING THE GROUPING OF BEATS WITH AGE ONSET OF HEART BEAT

END OF EMBRYONIC PERIOD



The above records, of 2-minute duration, were clipped from 10-minute records made at intervals of 6 hours. They represent successive stages in the development of the heart. The difference in length of these records may be accounted for by the differences in speed of the drum. The interval of time was recorded on the drum with the use of a Harvard timer.

VITA

Emily Walcott Emmart was born in Baltimore, August 8, 1898. After receiving her preliminary training in the schools of that city she began her academic education at Goucher College in 1918, receiving her A.B. degree in 1922. During the summers of 1921 and 1922 she took special courses in Invertebrate Zoology and Protozoology at the Marine Biological Laboratory, Woods Hole, Massachusetts. In the fall of 1922 she entered the graduate school of the Johns Hopkins University and began her studies for the degree of Master of Arts. She was awarded a graduate scholarship for the year 1923 to 1924, together with a research table at Woods Hole for the summer of 1923. Upon the completion of her thesis, "The inheritance of productivity in the Rotifer, *Distyla inermis*," she received the M.A. degree in the spring of 1924. From 1924 to 1928 she was Associate Professor of Biology at Western Maryland College. The summer of 1925 was spent traveling in Europe and the following summer studies were begun on the morphology of *Gammarus chevreuxi* at the Marine Laboratory, Plymouth, England. In 1927 she resigned from the faculty of Western Maryland College and returned to the Johns Hopkins to continue graduate work. The work on *Carinogammarus mucronatus* (Say) was begun and continued at Woods Hole during the summer of 1928 until December of that year. The dissertation was completed in the spring of 1930. After receiving the Ph.D. degree in 1930, she was sent to Mexico under the U. S. Department of Agriculture to investigate the cytological relationship of the various species of the Mexican Fruit Fly also known as the West Indian Fruit Fly, *Anastrepha*. She resigned and since then has been Instructor in Histology in the College for Teachers at the Johns Hopkins University.

